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Production and use of recombinant A β for aggregation studies

Tiernan T. O'Malley¹, Sara Linse², Dominic M. Walsh¹

¹Laboratory for Neurodegenerative Research, Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA;

²Department of Biochemistry and Structural Biology, Lund University, SE221 00 Lund, Sweden.

Abstract

The amyloid β -protein (A β) is believed to play a central role in Alzheimer's disease (AD) pathogenesis and there is great interest in understanding the process of A β aggregation, its underlying mechanism and the species generated during aggregation and their biological activity. Although A β has been studied for more than 30 years, analysis of its aggregation has been hampered by structural and chemical impurities. Here we provide a detailed protocol for the expression and purification of chemically and structurally homogeneous A β monomer. We also describe a method to produce covalent A β dimers linked by phenolic coupling of tyrosine residues.

Keywords

Alzheimer's disease; amyloid β -protein; dityrosine; dimer; fibrillogenesis; monomer; aggregation

1. Introduction

Aggregation of the amyloid β -protein (A β) is believed to play an initiating role in the pathogenic process that causes Alzheimer's disease (AD) (1). In vivo A β occurs as a family of related peptides with a common amino acid core of ~30 residues (reviewed in (2)). All members of the A β family are amphiphatic and have a proclivity to self-associate and aggregate often forming thermodynamically stable amyloid fibrils (3). Due to the high costs of synthetic A β systematic studies of aggregation and the use of peptides of varying primary sequences were limited. Moreover, sequence inhomogeneity in synthetic A β affects the underlying mechanism and overall rate of aggregation (4). Here we detail an inexpensive and simple system which can be used to generate naturally occurring A β primary structures (5–7) and A β containing designed mutations (8, 9). This method allows for the production of large quantities of chemically and structurally pure A β (5, 9) – prerequisites for kinetic and structural experiments. In addition, we have optimized a system to monitor A β fibrillogenesis which yields highly reproducible results (7, 10). We and other have used these advances to connect the macroscopic features of A β fibril formation to their microscopic determinants through the analysis of experimental kinetic data (11–14) and to study the structure of A β monomer and dimer (9).

Besides variations in A β primary structure, evidence suggests that post-translational modifications such as the formation of A β dimers may also be important in AD (2, 15–18).

In nature, there are few ways by which covalent A β dimers can be generated. The most likely mechanism of dimer formation involves coupling of two A β monomers by a dityrosine bond. Here we describe a method for the production of dityrosine A β that can be used to study kinetics of aggregation (9) and as a standard for use in immunoassays (19). In this chapter we describe protocols for preparation of A β 42 monomer and A β 42 dityrosine dimer, however, it is important to note that these methods are applicable to many other A β sequences. The establishment of a facile method to produce a multitude of highly pure A β peptides coupled with a robust protocol to monitor fibrillogenesis has already revealed important information (11–13) and offers a vehicle for further discovery when applied to the study of complex mixtures of A β species with different primary sequences and post-translational modifications.

2. Materials

2.1 Expression and purification of A β (M1–42)

1. Glycerol stock of chemically competent BL21* DE3 pLysS *E. coli* cells (Notes 1 and 2, 3).
2. pET-Sac vector containing a gene encoding for wild type, human A β (M1–42) (available from Addgene plasmid repository – www.addgene.org) (Note 3).
3. Agar plates containing ampicillin and chloramphenicol at a final concentration of 50 mg/l and 38 mg/l, respectively.
4. Autoclaved glass beads.
5. Sterile super optimal broth with catabolite repression (S.O.C.) (Thermo Fischer, Cat. #15544–034).
6. Lysogeny broth (LB) media containing ampicillin and chloramphenicol at 50 μ g/ml and 38 μ g/ml, respectively. LB (5 g NaCl, 5 g Bacto Tryptone and 2.5 g Bacto Yeast Extract) is made up to 500 ml using H₂O and autoclaved at 121°C for 21 min in a 2.5 L baffled flask. Smaller, 250 ml flasks containing 50 ml of LB are prepared for overnight starter cultures. Once autoclaved, the broth is allowed to cool to room temperature and 0.5 ml of a 50 g/l ampicillin solution (prepared in MQ H₂O and sterile filtered) and 0.5 ml of a 30 g/l chloramphenicol solution (prepared in 100% ethanol and sterile filtered) added to 500 ml of LB. Fifty μ l of chloramphenicol and 50 μ l of ampicillin solution are added to 50 ml of LB.
7. Induction solution: aqueous 1 M isopropyl β -D-1-thiogalactopyranoside (IPTG) stored as 1 ml aliquots at –20°C.
8. Preparation of DE23 anion exchange resin. Resin is resuspended in 0.1M HCl (15 ml per gram of dried resin), washed ten times with MQ H₂O, then once with 0.5 M NaOH, and finally ten times with MQ H₂O. The resin is allowed to settle between all steps and the supernatant decanted. The remaining base is neutralized by resuspending the resin in 250 mM tris containing 50 mM EDTA (unbuffered). Finally the resin is resuspended in 25 mM tris-HCl, pH 8.5

containing 5 mM EDTA at a 50:50 mix of buffer to resin. For long term storage, 0.01% w/v benzalkonium chloride is added and the resin kept at 4°C. Before use, resin is homogenized by gentle inversion and washed with 25 mM tris-HCl, pH 8.5 containing 5 mM EDTA until the pH of the flow through is 8.5.

9. Mixed bed resin (Sigma Aldrich, Cat. #M8032).
10. Buchner funnel and G3 filter paper (Whatman, Cat. #1003–917).
11. Anion exchange buffers: Buffer A - 25 mM tris-HCl pH 8.5 containing 5 mM EDTA. Low salt wash solution - Buffer A containing 25 mM NaCl, Elution solution – buffer A containing 50 mM NaCl, High salt wash solution – buffer A containing 500 mM NaCl, and High salt/urea regeneration solution – buffer A 500 mM NaCl and 8 M urea.
12. Inclusion body denaturant - Buffer A containing 8 M urea is used to solubilize bacterial inclusion bodies containing A β peptide. All urea containing solutions were deionized prior to using (Note 4).
13. Dialysis: Snake skin tubing, 3,500 MWCO (Thermo Fischer, Cat. #88242).

2.2 Size exclusion chromatography (SEC)

1. A Superdex 75 16/60 is used for purification of bacterial extract and two Superdex 75 10/300 columns linked in tandem for isolation of samples for aggregation assays. All columns are attached to a Bio-Rad Duo Flow FPLC eluted at 0.8 or 0.5 ml/min in a cold room at 4°C. Columns must be thoroughly cleaned before and/or after SEC using A β peptides (Note 6).
2. A β disaggregation solution: 50 mM tris-HCl, pH 8.5 containing 7 M guanidine HCl and 5 mM EDTA.
3. SEC elution buffer (for initial size purification): 50 mM ammonium, bicarbonate pH 8.5 – the pH of which is attained by dropwise addition of 28–30% ammonium hydroxide. Buffer is then passed through a 0.22 μ m filter and degassed under vacuum overnight.
4. SEC elution buffer (for aggregation studies): 20 mM sodium phosphate pH 8.0 containing 200 μ M EDTA and 0.01% sodium azide (depending on the experiment other buffers may be used, note 7). Phosphate buffer is prepared by combining 94.7 ml of a 200 mM Na₂HPO₄ solution with 5.3 ml of a 200 mM NaH₂PO₄ solution. To this, 0.4 ml of 500 mM EDTA and 1 ml of 10 % sodium azide solution is added and the solution made up to 1L with MQ H₂O. As with the preparative buffer, the experimental SEC buffer is passed through a 0.22 μ m filter and degassed as above.
5. Spectrophotometer and quartz cuvette.

2.3 Dityrosine cross-linking of A β (M1–42)

1. 10 mM hydrogen peroxide solution is prepared from a solution of 30% w/w (Sigma Aldrich Cat. #H1009; 8.82 M) by bring 56.5 μ L of stock to 50 ml using MQ H₂O.
2. Horse radish peroxidase (HRP): HRP (Thermo Fischer Cat. #31490) is dissolved in MQ H₂O to yield a 2 mg/ml solution.
3. Tissue culture incubator at 37°C.

2.4 Negative contrast Electron Microscopy

1. Dumont N4AC Tweezers (Dumoxel Biology, Cat. #72870-D).
2. Acrodisc Filters 33 mm/0.22 μ M (Millipore, Cat. #SLGP033RS). MQ water should be placed in a 5 ml syringe fitted with a 0.22 μ M filter.
3. EM grids: 200 mesh Copper Grids, Formvar/Carbon film (Electron Microscopy Sciences Cat. #EMS200-Cu).
4. 0.5% glutaraldehyde solution, prepared by dilution of aqueous glutaraldehyde EM Grade 8% (Electron Microscopy Science, Cat. #16020). Immediately before use a vial of 8% glutaraldehyde is removed from –80°C and diluted to 0.5% with MQ H₂O (6.25 μ L 8% glutaraldehyde in 10 ml MQ H₂O). Two ml of this solution is passed through a 0.22 μ m filter and collected into a clean eppendorf tube.
5. 2% (w/v) uranyl acetate stain (Electron Microscopy Sciences Cat# 22400). Dissolve 1 g in 25 ml of 100% ethanol and stir for 30 min. Shield solution from light with aluminum foil. Dilute to a final volume of 50 ml with MQ H₂O and store in 2, 50 ml tubes covered with aluminum foil.

3. Methods

3.1 Expression and purification of A β (M1–42) peptide

3.1.1 *E. coli* transformation

1. On ice thaw competent *E. coli* B121* DE3 pLysS cells (40 μ L cell suspension).
2. Thaw DNA plasmid stock from freezer.
3. Beside a Bunsen flame and using a sterile pipette tip, add 1 μ g of DNA to the *E. coli* suspension, gently flick tube to mix.
4. Incubate on ice for ~30 min.
5. Heat shock bacteria at 42°C for 45 sec and cool immediately on ice for 10 min.
6. Add 400 μ L of S.O.C. to bacterial suspension and incubate at 37°C for 30 min.
7. Transfer 50 – 100 μ L of bacterial suspension to an agar plate.
8. Add 5 – 10 sterile glass beads and shake several times to distribute cells.
9. Incubate overnight at 37°C (Note 8) in a dry incubator or warm room.

10. Plates with colonies can be used immediately or sealed with parafilm and stored at 4°C.

3.1.2 Colony selection and propagation

1. Sterilize and seal the tip of a glass Pasteur pipette using a Bunsen burner flame.
2. Use a sealed pipette tip to collect a small well-isolated single colony (Note 9).
3. Transfer the pipette to a 250 ml flask containing 50 ml of LB and agitate for 30 sec.
4. Remove and discard pipette.
5. Incubate the inoculated culture with agitation at 37°C overnight (max. 16 h).

3.1.3 Growth of transformed bacteria and induction of protein expression

1. Next day, determine the extent of bacterial growth in the starter culture by measuring the OD₆₀₀ (>2.0 desired).
2. Add 5 ml of starter culture to 500 ml LB media and agitate at 37°C.
3. Measure OD₆₀₀ at regular intervals until a value of ~ 0.6 – 1.0 (~ 2 – 2.5 h) is attained.
4. When OD₆₀₀ is ~0.6–1.0, add 125 µl 1 M IPTG solution (final concentration 400 µM).
5. Monitor OD₆₀₀ readings every 30 min until growth plateaus (~ 3 – 4 h).

3.1.4 Collection of bacteria

1. Harvest cells by centrifugation (10 min at 10,000 rpm; SORVAL-SLA-3000; 350 ml Sorvall centrifuge bottle).
2. Decant supernatant and scrape bacterial pellets into a 50 ml conical tube and suspend in 25 ml buffer A (per pellet from 1 L culture) and freeze overnight at –20°C.

3.1.5 Sonication and Isolation of Inclusion Bodies

1. Thaw pellet. Sonicate for 30 sec at 4 W and 30% amplitude (Branson digital sonifier model 250) and transfer to centrifuge tube.
2. Centrifuge suspension for 10 min at 18,000 rpm in a Sorvall SS-34 rotor.
3. Decant supernatant and resuspend pellet in 25 ml buffer A. Repeat steps 1–3 two times, i.e. a total of 3 sonication steps.
4. Resuspend the pellet in 12.5 ml inclusion body denaturant and sonicate for 30 sec at 4 W and 30% amplitude.
5. Centrifuge denatured inclusion bodies for 10 min at 18,000 rpm and 4°C in a Sorvall SS-34 rotor to remove debris.

6. Collect supernatant and store on ice.

3.1.6 Purification of A β (M1–42) from solubilized inclusion bodies

1. Dilute the 12.5 ml of denatured inclusion bodies with 37.5 ml of buffer A (1:4 dilution).
2. Add DE23 resin (Note 10) and incubate with gentle rocking for 30 min at room temperature.
3. Filter under vacuum using a Buchner funnel.
4. Wash the resin with 25 ml of buffer A, stir well.
5. Apply vacuum and collect the flow through.
6. Wash the resin with 25 ml of low salt solution and then elute with 4 $\times$ 50 mM NaCl in buffer A (Note 11).
7. Analyze a sample of each fraction using SDS-PAGE and silver staining (Note 12).
8. Pool A β containing fractions and transfer into 2 $\times$ 20 cm long pieces of snake skin tubing.
9. Dialyze 50 ml of pooled fractions into 5 L of 50 mM ammonium bicarbonate, pH 8.5, with three changes (to achieve a dilution of NaCl, tris and EDTA of 1¹⁰⁶).
10. Lyophilize samples and store at –80°C.

3.2 SEC purification of recombinant A β peptide

1. Dissolve the dried peptide at a concentration of ~ 10 mg/ml in 50 mM tris-HCl, pH 8.5 containing 7 M guanidinium HCl and 5 mM EDTA (7 M GuHCl) and incubate at room temperature overnight.
2. Inject 5 ml of A β solution onto a Superdex 75 16/60 column and elute at 0.8 ml/min in 50 mM ammonium bicarbonate, pH 8.5 (Note 13).
3. Monitor UV absorbance at 280 nm and collect the A β monomer peak in 1.2 ml fractions. Pool the peak fractions and measure the absorbance at 275 nm (Abs₂₇₅). The concentration of the A β can be determined using the extinction coefficient 1361 M^{–1} cm^{–1} (9) (Note 13).
4. Volumes containing 1 mg of peptide are frozen and lyophilized. Peptide mass and purity (> 99%) are assessed by MALDI-TOF mass spectrometry, reverse phase HPLC and SDS-PAGE with silver staining (Figure 1).

3.3 Preparation of dityrosine (DiY) cross-linked A β (M1–42)

1. Dityrosine cross-linking of peptides is performed essentially as described previously (9). Dissolve 5 mg of A β (M1–42) in 7 M GuHCl, incubate overnight at room temperature and purify by gel-filtration using two Superdex 75 10/300

columns linked in tandem and eluted in 50 mM ammonium bicarbonate, pH 8.5 (Note 13).

2. Collect 0.5 ml fractions, pool the A β monomer peak fractions, measure the Abs₂₇₅ and determine the concentration.
3. Make a 1:20 dilution of a 2 mg/ml HRP solution (2.2 μ M final concentration) and a 1:40 dilution of a 10 mM H₂O₂ (250 μ M final concentration) into the A β monomer solution. Adjust with 50 mM ammonium bicarbonate, pH 8.5 to give a final A β concentration of 40 μ M and incubate at 37°C for ~14 h (20).
4. Quench the reaction on ice, freeze the solution and lyophilize. Dissolved the lyophilizate in 7 M GuHCl (3 ml) and incubate overnight at room temperature.
5. Isolate dityrosine cross-linked A β dimer ([A β (M1–42)]_{DiY}) from unreacted monomer, HRP and aggregated A β using a Superdex 75 16/60 column eluted with 50 mM ammonium bicarbonate, pH 8.5 (Note 13).
6. Collect 0.5 ml fractions, pool dimer containing peak fractions and determine concentration by measuring the absorbance at 283 nm and using the extinction coefficient for dityrosine cross-linked A β , ϵ = 6226 M⁻¹ cm⁻¹ (9).
7. The above protocol typically yields 1 mg of [A β (M1–42)]_{DiY} for every 4 mg of starting A β (M1–42) monomer.

3.4 Continuous Thioflavin T dye binding assay

1. ThT assay is performed essentially as described previously (8). Dissolve 1 mg of A β monomer or dimer peptide in 0.55 ml 50 mM tris-HCl, pH 8.5 containing 7 M GuHCl and 5 mM EDTA and incubated overnight at room temperature.
2. Centrifuge at 16,000 g for 30 min prior to injection onto SEC.
3. Inject 0.5 ml of peptide solution onto two Superdex 75 10/300 columns linked in tandem and elute in 20 mM sodium phosphate, pH 8.0 containing 200 μ M EDTA and 0.02% NaN₃. Collect 0.5 ml fractions.
4. Prepare a working stock of SEC isolated peptide at 10.1 μ M with 20 mM sodium phosphate, pH 8.0 containing 200 μ M EDTA and 0.02% NaN₃ (see step 2.2.5.).
5. A working stock containing ThT is prepared by combining 1980 μ l of 10.1 μ M A β solution with 20 μ l of 100 $\times$ ThT stock (2 mM), to yield 20 μ M ThT and 10 μ M A β .
6. Prepare serial dilutions of the A β ThT solution by combining 1000 μ l of solution containing 20 μ M ThT with 1000 μ l of dilution buffer to yield a 1:2 dilution and make further dilutions as required.
7. Using reverse pipetting technique, transfer 6 $\times$ 100 μ L of each A β concentration to the wells of a black, clear bottom, 96 well half-area low-binding polystyrene plate.

8. Include a blank without any A β (i.e. dilution buffer alone) alongside A β containing wells. The outer 36 edge wells of the plate are not used for samples, but are filled with buffer and plates sealed using a low fluorescence adhesive cover.
9. Incubate samples at 37°C in a CLARIOstar plate reader and record the fluorescence at 5 min intervals (Ex_{440nm}; Em_{480nm}).

3.5 Negative contrast Electron Microscopy

1. Transfer 10 μ l of sample to duplicate 200 mesh, formvar coated, copper electron microscopy grid (Electron Microscopy Sciences, Cat. #EMS-200-Cu). Incubate for 60 sec.
2. Add 10 μ l of 0.5% glutaraldehyde solution and incubate for exactly 60 sec.
3. Careful wick solution onto blotting paper making sure not to touch the face of the grid.
4. Wash the grid with 3 drops of MQ H₂O passed through a syringe fitted with a 0.22 μ m filter. If necessary, carefully wick solution onto blotting paper (Note 14).
5. Add 10 μ l of uranyl acetate solution to the grid, and incubate for exactly 120 sec. Thereafter, carefully wick solution onto blotting paper.
6. Allow to dry for at least 5 min.
7. Grids are visualized using a Techni G² BioTWIN electron microscope (FEI). Scan the grid at ~ 12,000 magnification, in a serpentine fashion making note of the relative abundance and morphology of species seen.
8. Representative images (Figure 2) are taken of several fields using an AMT 2k charged coupled-device camera.

4. Notes:

Note 1: All steps involving bacteria are performed using standard aseptic techniques.

Note 2: Preparation of chemically competent BL21* DE3 pLysS *E. coli* cells: add 1 μ l of stock BL21* DE3 pLysS cells (Promega Cat. #L1195) to 50 ml of autoclaved LB media containing 38 μ g/ml of chloramphenicol. Incubate with shaking at 37°C until OD₆₀₀ reaches ~ 0.6. Thereafter, transfer cells to a sterile 50 ml tube and centrifuge at 2500 rpm (Eppendorf 5810R centrifuge with A-4-81 rotor) for 10 min at 4 °C. Decant supernatant and gently resuspend the pellet in 20 ml of ice cold Tfb1 buffer*. Leave cells stand on ice for 10 min and repeat centrifugation. Decant supernatant and gently resuspend cells pellet in 2 ml of ice cold Tfb2**. Prepare 40 μ l aliquots and store at -80°C.

* Tfb1 contains 30 mM potassium acetate, 50 mM manganese chloride, 100 mM potassium chloride, 10 mM calcium chloride, 15% glycerol and the pH is adjusted to 5.8 with 0.1 M acetic acid. ** Tfb2 contains 10 mM sodium-MOPS, 75 mM calcium chloride, 10 mM potassium chloride, 15% glycerol and the pH adjusted to 7.0 with 0.1 M NaOH.

Note 3: A number of *E. coli* strains are suitable in which to express A β . For instance, for A β (M1–42) we have used *E. coli* strain BL21* DE3 PLysS, and for A β (M1–40) we have used *E. coli* strain T7 Express (C2566, New England BioLabs Inc, Ipswich, MA, USA, previously called ER2566). To ensure optimal and reproducible yields of other A β variants it may be necessary to test expression in a selection of *E. coli* strains.

Note 4: The gene encoding A β 42 contains an AUG “start” codon which gives rise to a methionine that proceeds the Asp1 start site of A β , therefore we refer to this form of A β as A β (M1–42) - the sequence of which is:
MDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGGVIA.

Note 5: Solutions of urea may contain iso-cyanic acid, a thermal breakdown product of urea that can cause carbamylation of primary amines in proteins including A β . Therefore all solutions containing urea are prepared just prior to use and deionized using mixed bed resin.

Note 6: SEC columns are washed as described previously (21). Columns are transferred from elution buffer into MQ H₂O, washed with 1 column volume (CV) of 50% formic acid; 2 CV of MQ H₂O; 1 CV 0.5M NaOH; 2 CV MQ H₂O; 1 CV 1M unbuffered tris; 2 CV MQ H₂O, and stored in 20% ethanol.

Note 7: The last gel filtration step is performed using the desired experimental buffer as running buffer. However, for studies under conditions where A β is more aggregation prone, it is recommended to perform SEC in a “low aggregation buffer” and adjust the solution composition after SEC isolation. For example, when lower pH than 8 is desired, gel filtration is performed at pH 8, and the pH is adjusted afterwards while the monomer is kept in an ice box. When higher salt concentration is desired, gel filtration is at moderate ionic strength (e.g. in 20 mM sodium phosphate or other buffer of choice), and salt is added from a concentrated stock to the isolated monomer in an ice bath.

Note 8: Typically, we obtain 25–50 small, round colonies distributed evenly over the agar surface.

Note 9: It is important to pick the smallest well-isolated colonies. These are the ones most likely express high levels of A β . Do not add bacteria from more than one colony in each flask; a low-level expressing clone will tend to grow fast and take over the culture at the expense of higher expressing clones that tended to grow more slowly. If the intention is to grow multiple 500 ml day cultures, use several colonies in individual over-night cultures. Even though BL21 DE3* pLyS tends to give very uniform expression levels, this limits losses that would arise should a single colony be used that may a low-level expresser.

Note 10: DE23 anion exchange resin is prepared as a 50% slurry in buffer A. Apply 25 ml of slurry to a Buchner funnel and vacuum dry. To equilibrate the resin and remove residual benzalkonium chloride, rinse x5 with 25 ml of buffer A.

Note 11: Ion exchange resin may be regenerated by washing with high salt buffer and then high salt/urea regeneration buffer.

Note 12: A β monomer migrates at approximately 4.5 kDa on 16% polyacrylamide tris tricine gels. Using the silver staining protocol of Shevchenko et al., it is possible to readily detect 100 ng of A β (5, 22).

Note 13: A β monomer elutes from a Superdex 75 16/60 column at ~83 ml, whereas dimer elutes at ~74 ml (peak volumes). Using two Superdex 75 30/300 columns linked in tandem, A β monomer elutes at ~24.5 ml and dimer at ~21 ml (peak volumes). Depending on batch size, larger columns may be used, but when larger columns are employed A β monomer and dimer will elute in larger volumes.

Note 14: Rotate grid 90° and slowly push a drop of MQ H₂O from a syringe with filter attached over the grid and allow it to fall onto blotting paper below. Repeat this three times.

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Figure 1. Analysis of peptide purity and mass.

The purity of recombinantly produced $A\beta$ is assessed by (A) reverse phase HPLC and (B) SDS-PAGE with silver staining. For RP-HPLC analysis of each peptide, 25 μ l of a ~ 0.2 μ g/ μ l solution (in 0.1% trifluoroic acid; TFA) is injected onto a Capcell CN column and eluted with a linear gradient of 5–65% acetonitrile in 0.1% TFA over 35 min (A). The same sample preparation (12 μ l) was treated with 12 μ l of 2 $\times$ sample buffer and duplicate 10 μ l lots (~ 1 μ g) electrophoresed on a 16% polyacrylamide tris-tricine gel. Gels are visualized with silver staining. (C) Peptide mass is determined by ion-trap mass spectrometry using a Q Exactive hybrid quadrupole-orbitrap connected to a Dionex 3000 LC eluting from 100% buffer A (0.075% NH_4OH in 5% acetonitrile/ H_2O) to 50% buffer B (0.025% NH_4OH in 95% acetonitrile/ H_2O) over 20 minutes. Monoisotopic mass spectra are given for the most abundant isotope and charge state present ($m/z_{A\beta(M1-42)} = 4+$, Expected $Mr_{A\beta(M1-42)} = 4644.30$, Observed $Mr_{A\beta(M1-42)} = 4644.16$; $m/z_{[A\beta(M1-42)]_{DiY}} = 6+$, Expected $Mr_{[A\beta(M1-42)]_{DiY}} = 9286.58$, Observed $Mr_{[A\beta(M1-42)]_{DiY}} = 9286.60$).

Figure 2. Both Aβ(M1-42) monomer and dityrosine dimer aggregate to form Aβ fibrils. SEC-isolated Aβ(M1-42) and [Aβ(M1-42)]_{DiY} was diluted to 10.1 μM with 20 mM sodium phosphate pH 8.0 containing 200 μM EDTA and 0.02% NaN₃. Six, 100 μl replicates of each peptide were transferred to a 96 well, half-area plate incubated at 37°C for 18 hours. Peptide samples were analyzed by negative-stain electron microscopy. Size bars = 100 nm.